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Role of Cortisol in Serum and Follicular Fluid in Sub Fertile Women Undergo Intracytoplasmic Sperm Injection

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Abstract **Background:** Cortisol, is a glucocorticoid hormone produced by the adrenal glands, performs many vital functions in the body, including the regulation of energy, metabolism, and immunity. The body also releases cortisol to activate our "flight-or-fight" responses to counteract acutely stressful situations. If cortisol levels stay elevated for extended periods, however, they can lead to chronic stress. This long-term activation of the stress-response system can interfere with the normal functioning of other body systems, including reproductive health. Several studies demonstrate a relationship between stress, cortisol and infertility – higher levels of stress and cortisol result in a decreased fertility rate [1]. **Aim of the study:** to detect the follicular fluid and blood concentration of the cortisol hormone on subfertility in women undergoing Intracytoplasmic Sperm Injection (ICSI) for fertility treatment. **Method:** This case control study carried out on eighty women, from these, forty female were subfertile patient defined as the cases, other fourty female as control included fertile female came to fertility center due to male facter of subfertility. A detailed medical history, physical examination was recorded. At the same day of ovum pickup blood and follicular fluid were collected, for hormonal analysis of cortisol. **Results:** Show significantly higher levels of serum and follicular cortisol in subfertile women (cases) than control group ($p < 0.05$), and significant positive correlations between serum and follicular fluid. **Conclusion:** Cortisol hormone level in blood and follicular fluid were significantly higher in cases group than control and there is a positive correlations between serum and follicular fluid of cortisol.

Key Words steroid hormones, cortisol, follicular fluid, female infertility, intra cytoplasmic sperm injection

1. Introduction

It is estimated that approximately 10-15% of couples world-wide experience subfertility [2], and defined as the inability of a couple to achieve pregnancy over an average period of one year (for women under 35 years of age) or six months (for women above 35 years of age) despite engaging in adequate, regular (3-4 times per week), unprotected sexual intercourse [3]. So, it is a complex disorder associated with significant medical, psychosocial, and economic challenges, this condition can be attributed to factors related to the woman, the man, or both, and it is further classified into primary or secondary subfertility. Primary subfertility refers to couples who have never been able to conceive, while secondary subfertility involves difficulty in conceiving after having previously conceived, whether the pregnancy was carried to term or ended in a miscarriage. [4] management of for subfertility is tailored to the underlying cause and can be categorized as medical or complementary and alternative therapies. The choice of treatment may involve a combination of methods [5].

Subfertility treatment strategies are improving, with a more rapid transition to assisted reproductive technology (ART) treatments after unsuccessful non-ART treatments. This trend increases the potential importance of intracyto-plasmic sperm injection (ICSI) of subfertility treatments [6]. Follicular fluid provides a very important microenvironment for the development of oocytes and consists of a plasma transudate and follicular secretions. Thus, alterations in the composition of FF may directly affect follicular development and oocyte maturation The follicular fluid produced by gran-ulosa cells, and is composed of metabolic products, steroid hormones, polysaccharides, proteins, reactive oxygen species (ROS), and antioxidants that affect folliculogenesis. [7] [8]. This review provides an overview of the current knowledge about the biochemical predictors of oocyte quality in FF [9]. The physiology of the steroid hormone cortisol which

plays an important role in numerous processes including metabolism, blood pressure, and immune response regulation, and thus has proved a reliable biological correlate of many adverse health outcomes [10].

In the context of IVF, a growing body of evidence suggests that stress may exert its deleterious effects on IVF treatment outcomes through activation of the HPA axis, this hypothesis is plausible because both physical and emotional stress can cause alterations to the endocrine axis which may, in turn, affect the reproductive system through immunosuppression. Our study examines the relationship between serum and FF cortisol levels and fertility.

2. Methods

Case control type which was held in fertility center of Al-Sader medical city and from fertility center in Al-kafeel Hospital in Karbala city between January, 2022 and February, 2024. It included a total number eighty female with random sampling method, the age of these women ranging between (19-40) years with different ranging BMI, with duration of subfertility between (1-23) years, all are reside at middle Euphrates region in Iraq, from these eighty women, forty female were sub fertile patient defined as the cases, half of these cases have polycystic ovaries whom diagnosed according to history, clinical examination, ultrasound and hormonal level and the other half of cases are non-polycystic ovary. The other forty female (the control group) included fertile female came to fertility center due to male factor of subfertility with matched age and body weight.

A. Blood samples collection

On the same day of ICSI, few milliliters of venous blood were drawn by disposable needle and plastic syringes and distributed in a gel tube from each women in both groups, the cases and the controls for each women. The blood sample was collected and kept frozen at minus 20 degrees centigrade until all of the samples were collected for analysis and measurement of the hormone's cortisol. The blood was then centrifuged at roughly 3000 rounds per minute for 5 minutes.

B. Follicular fluid collection

Participating in this study were all subfertile women (disease and control) who underwent controlled ovarian hyperstimulation using a short protocol (antagonist cycles) in which a dialy dose of GnRH antagonist was given along with gonadotrophin stimulation. After that, the women received a HCG injection to induce ovulation, and a transvaginal Wallace needle was used under ultrasound guidance to collect the ova under general anesthesia. The follicles were measured and estimated to be between 16 and 22 mm in diameter. The ova and follicular fluid were next separated and placed in specialised media. After then, ten millimeters of follicular fluid were gathered and spun at 3000 rounds per minute for five minutes. The FF that had separated was then placed into doposable plain tubes and kept at 20 minus degrees Celsius for further examination and hormone measurement. In the meanwhile, the embryologist removed the cumulus cells from the ovum in order to inject sperm intracytoplasmically.

C. Statistical analysis

Statistical analysis was done using version 23 of SPSS (statistical package for social sciences). Among which mean and standard deviation are descriptive statistics. Applications for the analysis were the independent sample t-test and the pearson correlation coefficient. A significant P value is any value less than 0.05.

D. Ethical consideration

The participant received their informed, free will permission (the diseases and controls). Every participant received thorough information on the questions to be answered, the intended use of the gathered information, and the need of maintaining the privacy of their personal information. The process of drawing and sampling blood was thoroughly explained. With a clear explanation of the research's goal, the participants were made aware of their rights and allowed to withdraw at any moment.

E. Biochemical analysis

Serum and follicular level of steroid hormone Cortisol analysis using enzyme-linked immunosorbent assay "ELISA" system "Bio Tek" is used. Human –type kit from (Elabscience-USA) were applied for detection of Cortisol level in serum and follicular fluid.

3. Results

A. Comparison between cases and controls regarding age, duration and BMI

A total of 80 sub fertile women had been included in this study. Half of them due to male factor (controls) and the other half were female factors (cases). The female factors were PCO (N=20) and non PCO (N=20). The mean age of all women was 29.5±6.8 years(19-40) and the mean duration of infertility was 7.16±4.6 years(1-23). Table 1 shows some sociodemographic characteristics in which there is no significant difference between cases and controls regarding age, duration of infertility and body mass index of participants (p value>0.05).

B. Comparison of Serum hormone Cortisol in cases and controls

Table 2 compares the serum hormones of the cases and controls. The data compares serum cortisol, between cases and controls. The serum cortisol levels is significantly higher in cases as compared controls (p value<0.05).

C. Comparison of follicular fluid hormone Cortisol in cases and controls

Table 3 Comparison of follicular fluid hormone Cortisol in cases and controls FF hormone cortisol presented in Table 3 shows significantly higher levels in cases compared to controls with p<0.05

Variables	Cases (n=40) mean±SD	Controls (n=40) mean±SD	P value
Age/years	30.9±6.9	28.12±6.5	0.070
Duration/years	7.32±4.2	7±4.90	0.755
BMI Kg/m2	26.6±5.36	27.89±3.78	0.241

Table 1: Comparison between cases and controls regarding age, duration and BMI

Variables	Cases (n=40) Mean±SD	Controls (n=40) Mean±SD	P value
S. cortisol ng/dl	39.91±16.40	5.40±2.12	<0.001

Table 2: Comparison of Serum hormones in cases and controls

Variables	Cases (n=40) Mean±SD	Controls (n=40) Mean±SD	P value
F. cortisol ng/dl	16.17±6.60	5.78±2.55	

Table 3: Comparison of FF hormones in cases and controls

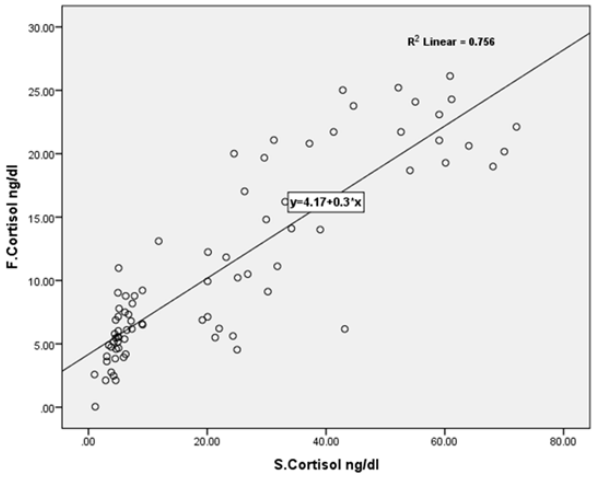

Figure 1: Significant positive correlation between serum and FF cortisol levels

D. Comparison between PCO and Non PCO patients in serum hormones

Regarding cases with female factors due to PCO or non PCO causes, Table 4 compare between the two groups and the result were significantly higher level in PCO women compared to non PCO causes.

E. FF hormone comparison in PCO and non-PCO patients

Tables 5 compare PCO and non-PCO that showed causes in FF cortisol which also were significantly higher in PCO patients compared to non PCO.

F. Correlation between serum and follicular hormone among cases

Tables 6 present statistically significant correlations between serum and FF hormone cortisolamong cases; Figure 1 demonstrate a positive significant correlation between serum and FF hormones, cortisol among all participants.

4. Discussion

Regarding some sociodemographic characteristics Table 1 age, duration of subfertility and body mass index, in which there is no significant different between case and controls between all participants, case and control (P value > 0.05).

The results of study in the Table 2 and 3 show a significantly higher levels of serum and follicular steroid hormones Cortisol in sub fertile women (cases) than control group. Cortisol level elevation in infertile women could be part of stress hormone disturbances which have direct effect on FSH and LH production.

The same results was obtained by several studies have been published, by Wdowiak et al. [11], Atalyan et al. [12], Wdowiak A, et al, 2020 [13]. Studies revealed that infertile women had significantly higher cortisol levels than fertile women. While Miller et al. [14] that high FF cortisol levels increase fertilization rates, stress does not negatively affect the success of IVF. However, a number of research indicate that cortisol has no significant impact on oocyte maturity or fertilization rates in stimulated and unstimulated ICSI cycles.

These studies include those by Matzavinos et al. [15] and Csemiczky G. et al. [16].

Because increasing evidence indicates that higher steroid hormones, especially androgen levels, are a fundamental factor in the pathogenesis of PCOS [17] , Qi et al. [18] that PCOS patients with insulin resistance (IR) had elevated cortisol levels and decreased endometrial insulin sensitivity in comparison to non-IR patients. Wu et al. [19] that increased cortisol levels in the follicular fluid (FF) were associated with granulosa cell (GC) insulin resistance and metabolic disorders in PCOS. We hypothesize that PCOS patients whose cortisol levels are maintained at an optimal level may experience embryo implantation benefits.

The results shown in Table 6 and Figure 1 show a significant positive correlations between serum and follicular fluid steroid hormones Cortisol, between cases and controls. Therefore, it could be a useful predictor of oocyte maturation as well as fertilization rate for sub fertile women undergoing ICSI. This study's findings support those of Teissier et al.

Serum Hormones ng/dl	PCO (n=20) Mean±SD	non PCO (n=20) Mean±SD	P value
Cortisol	50.63±12.99	29.20±11.91	<0.001

Table 4: Comparison between PCO and Non PCO patients in serum hormone

FF Hormones ng/dl	PCO (n=20) Mean±SD	non PCO (n=20) Mean±SD	P value
Cortisol	20.13±3.58	12.20±6.59	<0.001

Table 5: Comparison between PCO and Non PCO patients in FF hormone

Correlations		r	P value
S. Cortisol ng/ml	F. Cortisol ng/ml	0.719	<0.001

Table 6: significant positive correlations between serum and FF hormones among cases

[20], who proposed that serum and follicular steroid content, follicular size, and oocyte maturity have a correlation that may affect the outcome of oocyte fertilization during ICSI.

5. Conclusion

1) Cortisol hormone level in blood and follicular fluid were significantly high in cases (sub fertile women), while significantly lower in control (male factor sub fertile).
2) There is a significant correlation between serum and follicular fluid Cortisol.
3) The importance of psychological stress in sub fertile female undergo intracytoplasmic sperm injection (ICSI).

Conflict of interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

All authors contributed equally in this paper.

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